percutaneous absorption and distribution of 2-naphthol

h. g. w. m. hemels

PROMOTORES

PROF DR J W H MALI

EN

PROF DR J M VAN ROSSUM

PERCUTANEOUS ABSORPTION AND DISTRIBUTION OF 2-NAPHTHOL

PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE
WISKUNDE EN NATUURWETENSCHAPPEN
AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS
DR CH M. A. KUYPER
HOOGLERAAR IN DE FACULTEIT DER WISKUNDE EN NATUURWETENSCHAPPEN,
VOLGENS BESLUIT VAN DE SENAAT
IN HET OPENBAAR TE VERDEDIGEN OP
VRIJDAG 24 NOVEMBER 1972 DES NAMIDDAGS TE 4 UUR

DOOR

HENDRIKUS GERHARDUS WILHELMUS MARIA HEMELS

GEBOREN TE HEINO

1972
DRUKKERIJ-UITGEVERIJ BRAKKENSTEIN TE NIJMEGEN

Mijn gedachten gaan uit naar allen die mij, bij de totstandkoming van dit proefschrift, op enigerlei wijze tot hulp en steun zijn geweest Hiervoor betuig ik mijn oprechte dank
Dit proefschrift is tot stand gekomen op de afdeling Dermatologie van de Katholieke Universiteit te Nijmegen Medewerking verleenden de afdeling Farmacologie, Centraal Dieren Laboratorium, Medische Tekenkamer, Medische Fotografie en Klinische Farmacie
De uitgave van dit proefschrift werd mede mogelijk gemaakt door Schering Corporation U S A

Aan Ans, Willem en mijn ouders

CONTENTS

"Was man nicht hat, das eben braucht man;
und was man hat, das konnt' noch besser werden"

naar Goethe

INTRODUCTION

There can be little doubt, that 2-naphthol incorporated in a so-called "peeling paste", is the most effective peeling agent currently available It has been a major contribution to the management of persistant acne vulgaris since 1881

Yet, we must accept the fact that this drug has been shown to have a potential for harm It may be associated with formidable side effects, which are well known by the practioner Through skill gained from experience it has been possible to effectively manage most patients while avoiding serious complications

Frequently, the question arises regarding how much drug on what surface area can be safely applied An unequivocal answer is not yet possible As far as reliable information about absorption rate, distribution and metabolic fate of dermatotherapeutics is concerned, we have made little more than a beginning

The following chapters will deal with some of these problems

Chapter I, gives a short survey of the penetration through skin No attempt has been made to review the literature exhaustively There have been many reviews in recent years, the most complete of them are given as references in this chapter

Chapter II, describes a rapid procedure for determining 2-naphthol and its metabolites in plasma and urine Plasma levels and urinary excretion data for the drug are reported from patients treated with the peeling paste

Chapter III, reports the results of pharmacokinetic investigations on 2-naphthol in dogs Plasma levels and urinary excretion of the drug and its conjugates following a constant rate of intravenous infusion are given

Chapter IV, deals with the systemic absorption of 2-naphthol, measured as a function of time, drug concentration and ointment composition after application of the compound to the flanks of a dog

CHAPTER I

PERCUTANEOUS ABSORPTION

The development of an effective integument was a requirement forced upon nature by the emergence of life from the sea. Hence, the principal functions of the skin are to protect the body from physical and chemical insults and to help to maintain its internal environment. Therefore, it is designed to impede absorption.

Absorption is a dynamic process going on more or less simultaneously with a lot of other processes and cannot be considered in isolation. The epidermis is composed of cells in four stages of development before they are, finally, shed and replaced

It is taken for granted that the barrier is located in the keratinized cells of the stratum corneum, because the skin becomes increasingly permeable if successive layers are stripped by adhesive tape. Most water soluble, low molecular weight, non-electrolytes applied to the skin surface diffuse into the inner milieu approximately a thousand times more rapidly when the horny layer is diseased, damaged or removed.

In discussing percutaneous absorption it is necessary to consider the skin itself, as well as the drug and the vehicle in which the drug is presented to the skin surface

There are several possible routes for penetration through the skin; through the cells of the stratum corneum, through the hair follicles or sebaceous glands and through the sweat ducts. The contribution of the skin appendages to skin permeability is, usually, minimal owing to their relatively small total fractional area - approximately 1:1000 -. Theoretical considerations and some experimental results indicate that these shunt pathways can, however, be very significant during the first phase of the penetration for molecules that penetrate the stratum corneum slowly.

The literature on the influence of vehicles on skin penetration is confusing and often contradictory. Many skin applications have ordinary but complex vehicles included, e.g. emolients, emulsifiers, thickeners preservatives, substances to improve acceptability or stability. Such

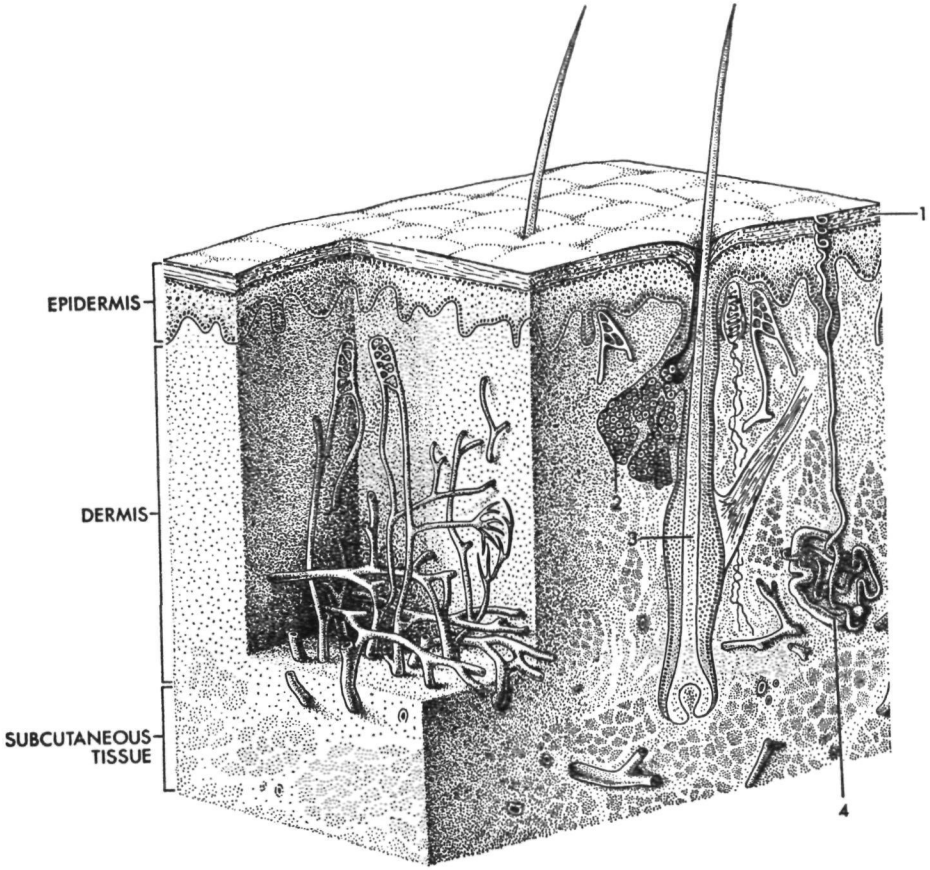


Fig. I-1. The stratified organization of the skin. The top layer, the epidermis, is thin but solidly cellular, in contrast to the much thicker and largely fibrous dermis which acts mainly as a support for the appendages, vessels and nerves. 1. Stratum corneum 2. Sebacecous gland, 3. Hair, 4. Eccrine gland.

substances sometimes interfere with efficacy by reducing the physiological availability of the active principle. The drug incorporated in the vehicle should be present in sufficient amount and reach the skin surface at an adequate rate. When the compound is insoluble, absorption is enhanced by reduction of particle size. In an emulsion, only the drug dissolved in the external phase is immediately available for diffusion.

The release of a drug is favoured by vehicles having a low affinity for the penetrant. The partition coefficient of the drug (and consequently

its thermodynamic activity) in the vehicle in relation to the skin, may vary as much as a thousandfold from one vehicle to another for identical concentrations Low partition coefficients are obtained when solutes are held firmly by the vehicle, for example in the cases where the drug forms soluble complexes with the vehicle The rate of release from such drug-vehicle combinations is slow, as was shown by Scheuplein and Blank in a study of the penetration of polar ethanol and nonpolar heptanol from water and lipid solvents In order to obtain maximum release rates each compound requires individual formulation based on solubility characteristics

It has often been stated that the addition of certain compounds may increase the absorption rate of drugs In many cases the acceleration is obtained by changing the polarity of the solvent or the vehicle in which the drug is incorporated

The acceleration may also be obtained by lowering the surface tension, which results in a better contact between the drug and the skin This surface tension lowering effect may promote the trans-appendage absorption Often the integrity of the skin barrier is affected more or less temporarily, for example by the dressing, by salicylic acid, some surface active agents, dimethylsulfoxide, etc

The absorption of a drug from a topically applied vehicle can be considered in two phases, primarily the drug penetrates from the surface of the skin into the living epidermal and dermal tissues and then secondarily into the circulatory system of the body Absorption in each of these two phases is referred to more specifically as **"intracutaneous"** absorption and **"systemic"** absorption respectively The degree of absorption in each of these phases can be varied dependent on the reason for initiating percutaneous absorption Generally, localized dermal therapy is desired and therefore an adequate amount of drug is intended to be concentrated in the intracutaneous phase at the specific site Subsequent systemic absorption should preferably be kept to a minimum in order to prolong the contact of the drug with the skin tissues and to reduce an eventual activity of the drug in causing undesirable systemic side-effects This is of particular relevance for potent dermatotherapeutics, e g phenols, corticosteroids

Quantitative data for the systemic absorption of these drugs are especially interesting for clinical management when these substances are applied to diseased or damaged skin, because under these circumstances the percutaneous absorption easily reaches a maximum, and moreover other influences may interfere

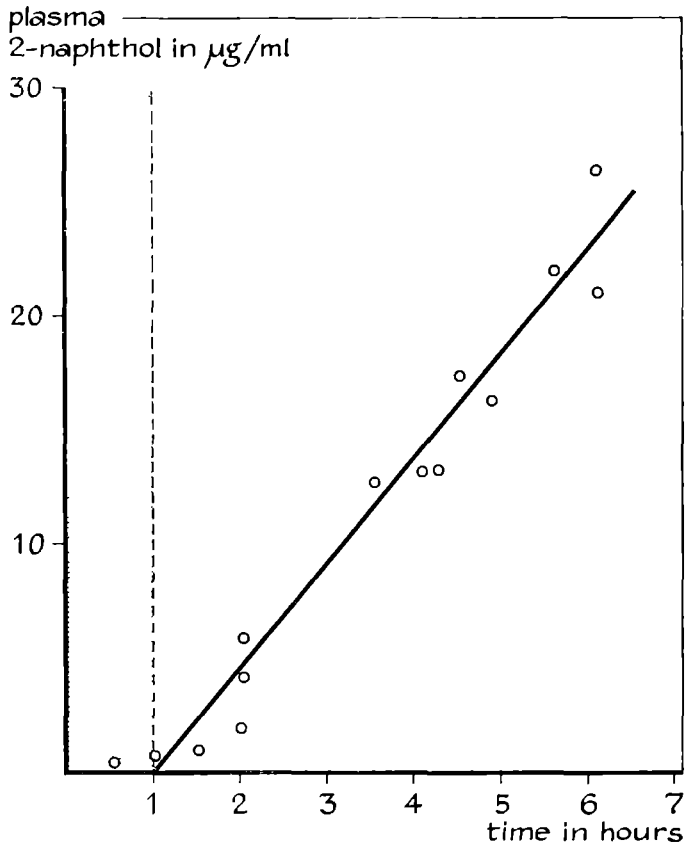


Fig I-2 Penetration of 2-naphthol through dog skin

Figure I-2 shows a typical curve for penetration of a non-electrolyte through the skin as a function of time.
Diffusion is virtually a passive physical process. The rates of absorption are determined by the physico-chemical properties of the absorbent and the membrane and by the concentration gradient of the absorbent through the membrane. The rate of absorption is given by Fick's general law of diffusion:

$$\frac{Q}{At} = J_s = K_p \Delta C_s$$

where Q is the amount of the penetrant; A is the area of the membrane; t is the time; J_s is the flux or the amount which penetrates per unit area

and per unit time, K_p is the permeability constant, and ΔC_s is the difference in concentration of the absorbent between both sides of the membrane

The amount of substance penetrating the skin plotted against time gives a straight line as soon as a steady penetration rate has been reached After the lag period the slope of this line is dependent on the concentration of the drug in the vehicle and the permeability of the membrane Consequently the plasma level curve of the drug after the lag time mimics a constant rate intravenous infusion

From a clinical point of view, in many instances, not only the total amount of drug reaching the systemic circulation is an important criterium but even more so is the rate at which the drug reaches the systemic circulation For the body level of the drug must be kept below the maximum level which might produce undesired effects and toxicity Since it is technologically infeasible to monitor the loss of drug from the skin to the absorption site, the rate of absorption derived from the drug's appearance in the equilibrated fluids of distribution of the body is a proper criterium for the rate of systemic absorption Now we are faced with a composite of two related research problems We must develop a highly sensitive and reasonably rapid analytical method for the determination of the drug and its metabolites in blood, urine and feces The development of this assay method will allow us to attack the second problem – the determination of the pharmacokinetic profile for the drug in an in vivo situation Determination of the pharmacokinetics following a constant rate intravenous infusion of the drug allows an appraisal of the penetration rate of the drug and an evaluation of the biological availability of the drug from different vehicles

REFERENCES

Caneghem, P. van (1969)
Penetration of drugs into the skin
Louvain Med **88** 11

Idson, B. (1971)
Biophysical factors in skin penetration
J Soc Cosm Chem , **22**, 615

Malkinson, F D., in Montagna, W. and Lobitz, W. C. (1964)
The Epidermis
New York The Academic Press

Munro, D., and Wilson, L., Editors (1969)
Transport Through the Skin
Br J Derm, **81**, suppl 4

Münzel, K. (1966)
Der Einflusz der Formgebung auf die Wirkung eines Arzneimittels
Basel Wissenschaftlichen Dienst Roche

Rossum, J. M. van (1971)
Significance of pharmacokinetics for Drug Design and the planning of dosage regimens
In Drug Design London Academic Press

Scheuplein, R. J. and Blank, I. H. (1971)
Permeability of the Skin
Physiol Review, **51**, 702

Tregear, R. T. (1966)
Physical Function of Skin
London Academic Press

Wagner, J. G. (1971)
Biopharmaceutics and relevant pharmacokinetics
Hamilton Drug Intelligence Publications

CHAPTER II

PERCUTANEOUS ABSORPTION AND DISTRIBUTION OF 2-NAPHTHOL IN MAN

SUMMARY

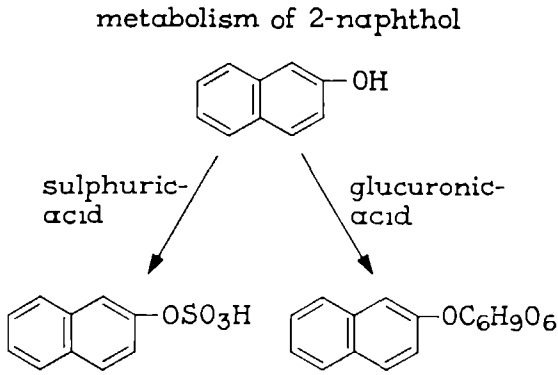
A procedure is described for determining unchanged ("free") and conjugated 2-naphthol in plasma and urine The method, based on gas chromatography, is rapid and has a limit of detection of approximately 2 µg/ml
The cutaneous barriers were found to be easily traversed by 2-naphthol Plasma levels of 2-naphthol and conjugated 2-naphthol during and after topical treatment with a 2-naphthol containing peeling paste were measured in four patients Plasma levels of "free" 2-naphthol were significantly lower than the plasma levels of conjugated 2-naphthol 24 *Hours urine excretion data of 2-naphthol following therapeutic application* of 2-naphthol containing ointment on the skin of ten patients are reported
The relevance of the data for clinical practice is discussed

INTRODUCTION

2-Naphthol (usually called beta naphthol) is a powerful dermatotherapeutic agent, which has been extensively used since its introduction by Ludwig and Kaposi in 1881 Before the advent of modern anthelmintics, 2-naphthol was also used as a vermicide, for which purpose it proved to have little therapeutic value because of its toxicity (Smillie, 1920) The effectiveness of 2-naphthol in peeling pastes for the treatment of persitant acne vulgaris has kept this drug in the current Pharmacopoeia's, although with restrictions as to its dosage (Volk and Winter, 1936, Percival, 1967; Powell, 1970)

The contents of this chapter will be published in Br J Derm 87 (1972)

In contrast to the large amount of data about side effects (Anon , 1922, Osol and Farrar, 1947, Albignente, 1968, Clarke 1969, Gleason, Gosselin, Hodge and Smith, 1969) there has been relatively little reported on the biochemical and pharmacological levels (Harkness and Beveridge 1966, Powell, 1970)
Administered to animals, 2-naphthol is excreted as a conjugate of glucuronic acid, as free 2-naphthol and to a minor extent as a conjugate of sulphuric acid (Lesnik and Nencki, 1886, Berenbom and Young, 1951, Boyland and Wiltshire 1953, Corner and Young, 1954, 1955)



This chapter will, therefore, deal with the development of a sensitive and rapid analytical method for the determination of 2-naphthol and its conjugates in plasma and urine

MATERIALS AND METHODS

Reagents and drugs

All reagents used were analytical grade (Merck), the drugs used were Dutch Pharmacopoeia, Edition VII, grade

Apparatus

A Hewlett Packard model F & M 402 high efficiency gas chromatograph, with a flame ionisation detector and a Hewlett Packard Moseley recorder model 7127 A, was used
The gas chromatograph was operated under the following conditions: U-shaped glass column (length 180 cm, inner diameter 0 3 cm) packed with 3 8%-UCW-98 on 80/100 mesh Chromosorb W (Dimethyldichlorosilane, high performance), nitrogen gas flow 50 ml/min, air flow 600 ml/min; hydrogen flow 50 ml/min, column temperature 160° C; injection port, flash heater and detector temperature 190° C

Extraction procedure

a The unchanged (free) 2-naphthol
Five ml urine or 2 0 ml plasma was acidified with 1 ml 1N hydrochloric acid and immediately thereafter extracted three times with 15 ml peroxide-free ether in a 40 ml glass-stoppered tube by shaking on an automatic shaker for 10 minutes. The ether extracts were dried with dehydrated sodium sulphate and evaporated to dryness in a 25° C water bath under nitrogen

b The total of free and conjugated 2-naphthol
Five ml urine or 2 0 ml plasma was acidified with 1 ml concentrated hydrochloric acid and the glass-stoppered tubes immersed in a waterbath at 70° C for 4 hours (Bakke and Scheline, 1969) The 2-naphthol was extracted as described above

Determination

The residue from the ether extraction was dissolved in ethyl acetate (between 0 10 and 5 00 ml, dependent on the concentration to be expected) and 3 microliter of this solution was injected into the gas chromatograph The height of the 2-naphthol peak on the chromatogram (for an example see figure II-1) compared with chromatograms obtained from known amounts of 2-naphthol was used as a measure of the amount of 2-naphthol in the sample Each extraction sample was run three times An average of the three determinations was taken. The sensitivity of the instrument was checked each hour with 2-naphthol

standard solutions at known concentrations Anthracene can be used as an internal standard

Patients and treatment

The subjects studied were hospitalized patients, aged from 18 - 22 years, under treatment for gross acne vulgaris, involving face, neck and trunk in various degrees The subjects were free of clinically demonstrable hepatic and renal diseases
The so called peeling paste applied, contained 20% 2-naphthol, 20% soft soap, 10% precipitated sulphur and 50% soft paraffin 7 5 gram of the ointment was applied daily to about 300 cm^2 of the skin with a wooden spatula, thereafter covered with bandages and left on the skin for approximately seven hours The applications were repeated daily until marked desquamation with inflammation resulted in removal of comedones and suppression of the acne

Urine and blood samples

a 24 Hours urine was collected during the hospitalization of ten subjects

b Both blood and urine samples of four subjects were taken on one or more days during the treatment period Venous blood samples were collected in heparinized tubes at the following intervals from the time of application of the peeling paste, 0, 1, 3, 8, 12, and 24 hours
Urine samples were collected coinciding with the time of blood sampling The free plasma and urine 2-naphthol was extracted during the day of collection The samples were stored in a refrigerator until used (4° C)

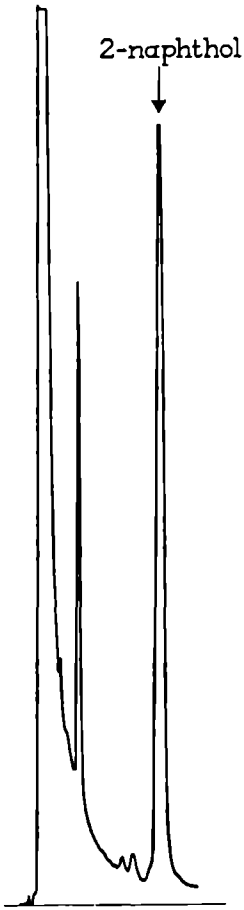


Fig II-1 Gas chromatogram of an extract from urine of a patient treated with a 2-naphthol peeling paste

RESULTS

Calibration curves and recovery data

The chromatograms of urine and plasma of control subjects showed no peaks interfering with the 2-naphthol peak. A gas chromatogram of patient's urine is shown in figure II-1. The results of the calibration of the chromatograph for 2-naphthol is shown in figure II-2. In order to check the recovery of 2-naphthol during the extraction procedure, known amounts of 2-naphthol were added to plasma and urine samples of control subjects. Tables II-1 and II-2 summarize the recovery data, which proved to be satisfactory.

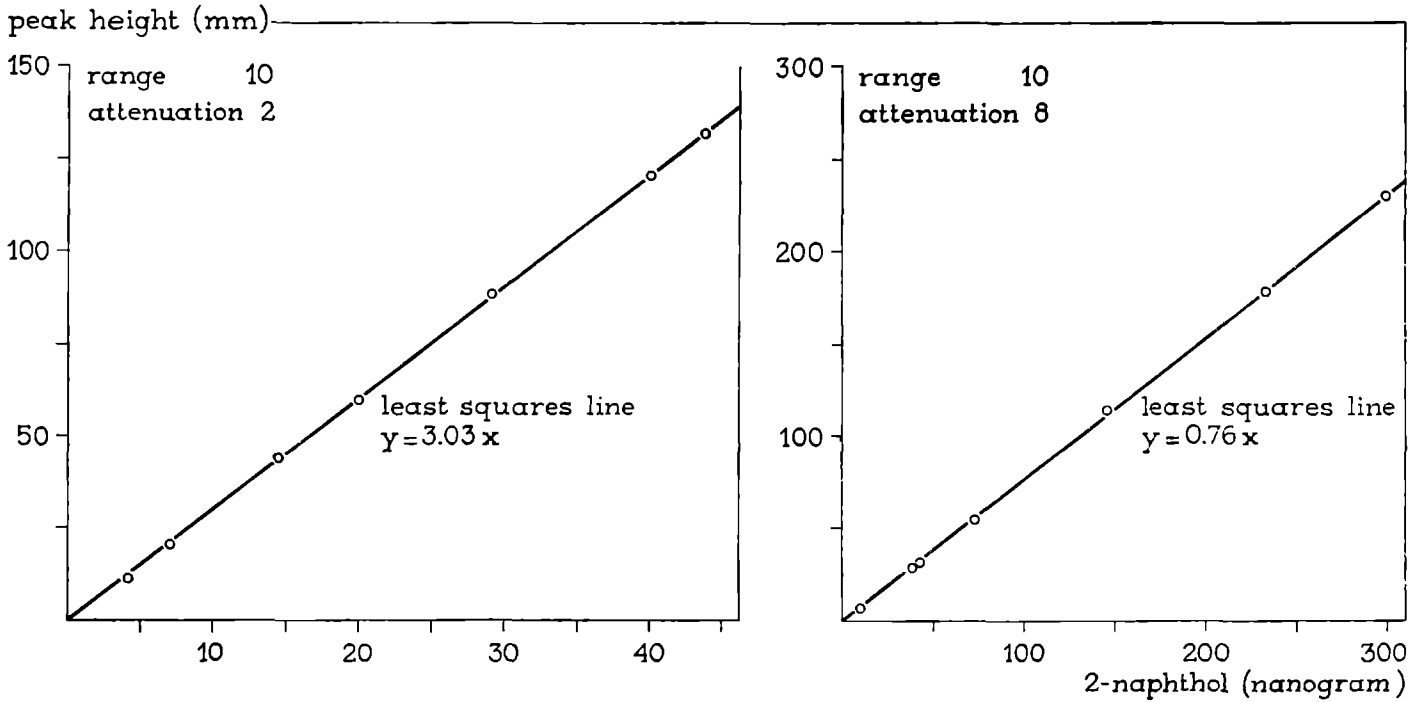


Fig. II-2. Calibration of the gas chromatograph by measurement of the peak height in mm from solutions with standard amounts of 2-naphthol in ng

Table II-1. Recovery data for 2-naphthol added to 5 ml urine samples.

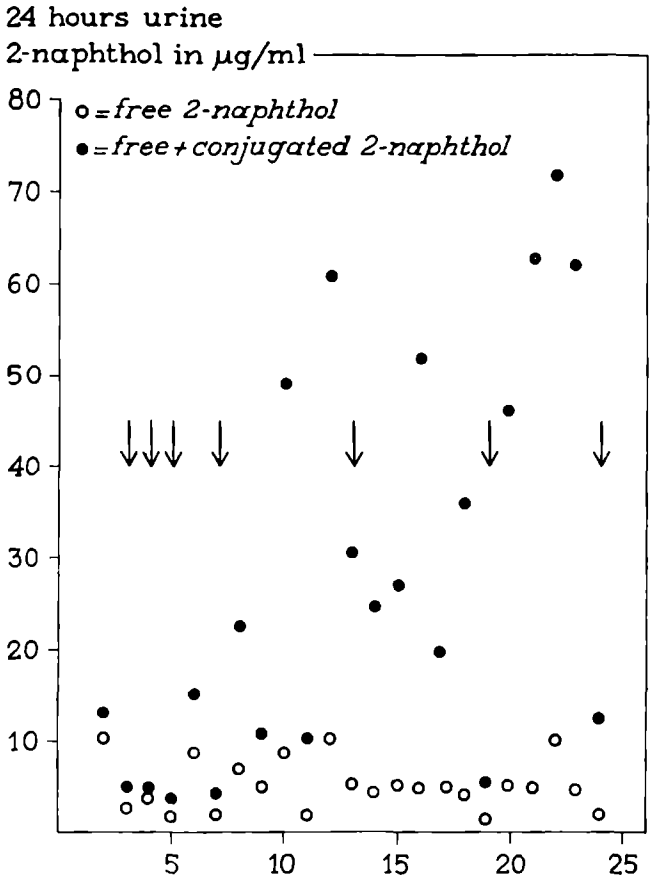
Amount of 2-naphthol added to 5 ml urine in micrograms	Recovered amount of 2-naphthol in micrograms	Percentage recovered
1.0	1.1	107
1.0	1.1	
10.0	10.2	102
10.0	10.1	
20.0	19.7	100
20.0	20.1	
50.0	50.1	100
50.0	49.5	

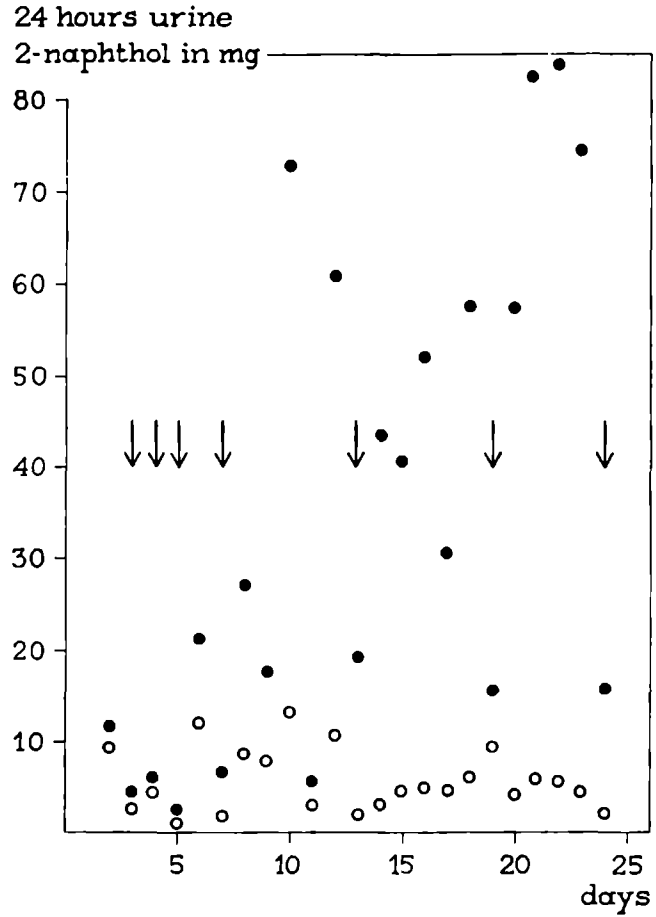
Table II-2. Recovery date for 2-naphthol added to 2 ml plasma samples.

Amount of 2-naphthol added to 2 ml plasma in micrograms	Recovered amount of 2-naphthol in micrograms	Percentage recovered
1.0	1.1	108
1.0	1.1	
10.0	9.4	95
10.0	9.5	

a. **24 Hours urinary excretion data**

The 24 hours urinary excretion data during 24 days of treatment of patient no 5 are shown as an example in figure II-3. Treatment of the back of the patients resulted in the highest amounts found in the urine. In figure II-3 the open circles in the range 35 - 37 $\mu g.ml^{-1}$ (50 mg - 146 mg) all represent values during the application of the naphthol ointment to the back of the patient.


24 hours urine
2-naphthol in µg/ml
o = free 2-naphthol
● = free + conjugated 2-naphthol
80
70
60
50
40
30
20
10
5
10
15
20
25


24 hours urine
2-naphthol in mg
80
70
60
50
40
30
20
10
5
10
15
20
25
days

24 Hours urinary excretion data for 2-naphthol and total (free + conjugated) 2-naphthol from 9 patients during the period of treatment - between 18 and 35 days - with 7 5 g 2-naphthol peeling paste are summarized in table II-3 The proportion of the amount recovered in the urine in relation to the applied daily dose of 1 5 g 2-naphthol, had a mean value of 5% (over 250 applications) with a maximum of approximately 41% The average percentage of free 2-naphthol present in the average amount of total excreted drug varied considerably in the patients, namely between extremes of 18% and 66%, the mean being 42% Patient no 10 is also mentioned but was treated with a very small amount of peeling paste

b **Combined plasma and urine analyses**

From figure II-4 it can be seen that the plasma levels of free 2-naphthol were different for the seven test days of three patients A slight rise in the plasma level was observed which reached a peak level 12 hours after application of the ointment The highest value was approximately 4 $\mu g\ ml^{-1}$ Two days after the last application the free 2-naphthol began to disappear from the blood The plasma level of free 2-naphthol was far less than the plasma level of conjugated 2-naphthol The plasma levels of conjugated 2-naphthol determined for the same seven test days are shown in figure II-5 In five tests the plasma levels of conjugated 2-naphthol reached peaks three hours after application of the ointment However in the other two tests, peak values were found eight hours after the application The highest mean peak level noted 3 and 8 hours after the application of the ointment were 23 0 $\mu g\ ml^{-1}$ and 21 7 $\mu g\ ml^{-1}$ Thereafter the levels decreased 24 hours after application of the ointment the mean value was 10 4 $\mu g\ ml^{-1}$ The amount of conjugated 2-naphthol in the plasma of 2 patients 48 hours after the last application was 3 6 $\mu g\ ml^{-1}$ and 3 2 $\mu g\ ml^{-1}$ respectively These values decreased further to 1 1 $\mu g\ ml^{-1}$ and 0 5 $\mu g\ ml^{-1}$ the next day The elimination rate constants r_{el}, of the conjugated 2-naphthol were obtained by means of graphical analyses of the plasma concentrations against time, using semilogarithmic graph paper (Van Rossum, 1971) The elimination rate constant, r_{el}, being the reciprocal of the elimination time constant τ_{el}, and the apparent biological half-life, $t_{1/2}$ ($t_{1/2}$ = ln

←

Fig II-3 Urinary free 2-naphthol and total (free + conjugated) 2-naphthol during hospitalization of patient no 5 Arrows indicate days of non-treatment

Patient	24 Hours Urinary Excretion of free 2-naphthol				24 Hours Urinary Excretion of total 2-naphthol				
	mean $\mu g.ml^{-1}$	range $\mu g\ ml^{-1}$	mean mg	range mg	mean $\mu g\ ml^{-1}$	range $\mu g.ml^{-1}$	mean mg	range mg	% free/total
1	21	4-47	25	8- 53	38	6- 97	51	12-146	61
2	27	8-48	36	13- 64	46	17- 73	58	24-146	66
3	16	2-40	17	3- 51	51	35-101	54	2-141	29
4	11	2-12	18	3- 61	20	8- 49	32	9- 76	55
5	6	2-11	6	2- 14	35	10-179	40	6-134	18
6	23	6-52	31	8- 58	77	25-229	95	36-309	35
7	15	2-39	23	2- 70	47	11-133	71	12-144	46
8	32	8-67	66	11-188	50	9- 99	102	14-278	48
9	13	5-23	26	11- 44	91	14-216	198	27-613	18
10	0.4	0- 3	5	0- 5	3	0- 54	4	0- 8	7

Table II-3

Urinary excretion data of 2-naphthol and total 2-naphthol (free + conjugated) from patients treated with 7.5 g peeling paste. Patient no 10 is also mentioned but was treated with a very small amount of paste

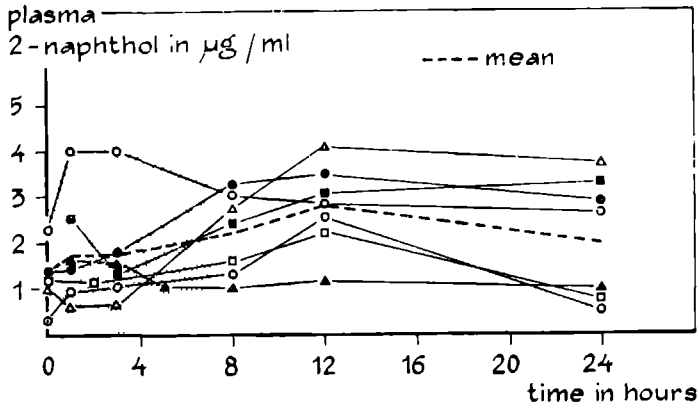


Fig II-4 Plasma levels of 2-naphthol in several patients during the 7 hours exposure to a 20% 2-naphthol peeling paste and 17 hours thereafter.

$2 \times \tau_{el}$) calculated for the conjugated 2-naphthol from 4 patients are summarized in table II-4.

The cumulative excretion data are shown in figure II-6. These urinary excretion data showed different values for each test day. The highest urinary excretion rates were observed during the first hours after application of the ointment. Unfortunately some urine samples were lost.

DISCUSSION

a. **24 Hours urinary excretion data**

The 24 hours urinary excretion data show that there is an average recovery of about 5% of the dose applied to 300 cm^2 of the body surface. Harkness and Beveridge (1966) reported the recovery in urine as about 10% of the total amount of 2-naphthol applied in a peeling paste to about 20% of the surface area of the subject. The difference in the results may very well have been caused by the different composition of the ointments used, (concentration, ointments base, etc.). Application of the ointment to the back of the patients

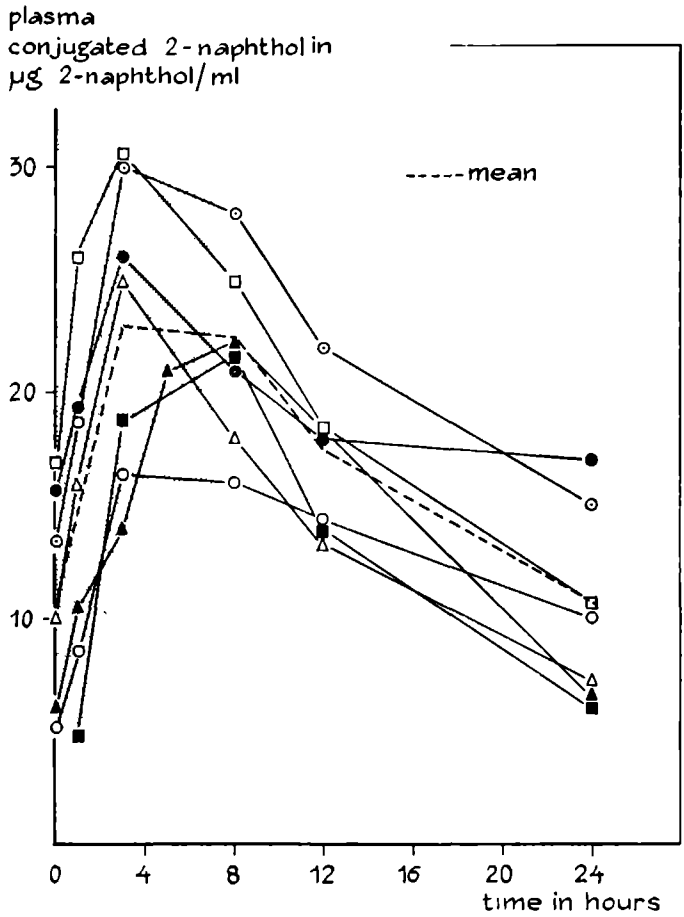


Fig. II-5. Plasma levels of conjugated 2-naphthol in several patients during the 7 hours exposure to a 20% 2-naphthol peeling paste and 17 hours thereafter.

resulted in the highest urine values. Here the application area may have been greater than is realized during the application of the ointment, because of squeezing and spreading under the dressings.
The amount of free 2-naphthol excreted in the urine as a percentage of the total amount excreted - mean value 41% - is similar to the 33-44% found in rats after subcutaneous injection of 2-naphthol (Berenbom and Young, 1951). There is a wide variation in these percentages of free 2-naphthol in our investigations. These different

Table II-4. Elimination rate constant, r_{el}, Elimination time constant, τ_{el}, and apparent biological half-life, $t_{1/2}$, of conjugated 2-naphthol in four patients (when the human body is regarded as a single-compartmental reservoir). - indicates: one day after cessation of therapy.

Patient	Day after start of therapy	Amount of paste applicated in g	Area of application (300 cm^2)	r_{el} in hr^{-1}	τ_{el} in hr	$t_{1/2}$ in hr
H	2	10	face	0.031	32.3	22.4
H	14	10	face	0.039	25.6	17.8
H	18	10	back	0.021	47.6	33.0
H	31	—	—	0.050	20.0	14.0
B	2	5	face	0.079	12.7	8.8
B	11	5	face	0.066	15.2	10.5
B	18	—	—	0.073	13.7	9.5
L	3	10	face	0.063	15.9	11.0
L	24	10	face	0.086	11.6	8.1
J	23	—	—	0.059	16.9	11.8

percentages of excreted free naphthol may have been caused by differences in metabolic capacity of the subjects and varying pH of the urine investigated. The passive tubular reabsorption of the unchanged drug, being a weak acid, will depend on the amount of lipid soluble, non-ionized naphthol present, which is strongly dependent on the pH of the urine. The maximum concentration found in urine was 67 µg free naphthol per ml urine.

b. **Combined plasma and urine analyses**

The clinical effect of the application of a 2-naphthol peeling paste (Powell, 1970) and the toxicological aspects of this treatment have been considered to result from the penetration of this drug through the skin. However, there has never been any reliable report on plasma levels of 2-naphthol. If we suspect the occurence of an intoxication due to this compound, we have to realize that the 2-naphthol plasma content will not give real information about the degree of exposure of the

subject to this drug The content of free 2-naphthol remains nearly constant at a low level soon after 2-naphthol has entered the circulation (figure II-4 and II-5) Besides, the ratio of free naphthol to conjugated naphthol in the plasma appeared not to be constant The appearance and occurence of conjugated 2-naphthol in the plasma is apparently "capacity-limited" in that it obeys zero order kinetics The 2-naphthol therefore seems to be readily absorbed and conjugated Conjugation and renal excretion of the unchanged drug seems to be the principal mechanisms of elimination.

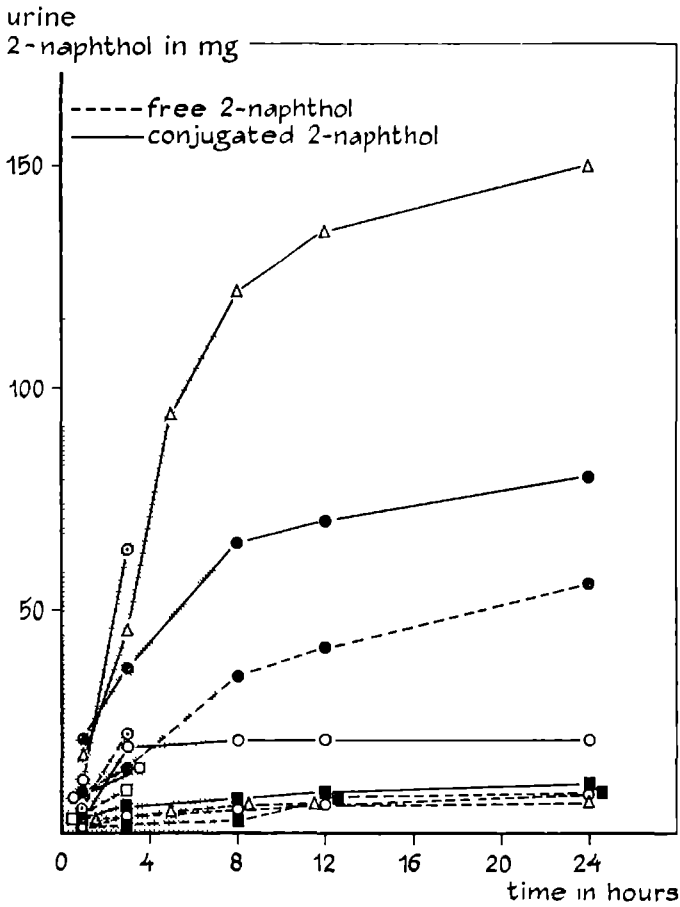


Fig II-6 Cumulative excretion of free 2-naphthol and conjugated 2-naphthol in the patients mentioned in fig II-4, II-5 and table II-4

The long-lasting plasma level of 2-naphthol is supposed to be caused by absorption from depôts upon, and in, the skin and other tissues, from tubular reabsorption and enterohepatic circulation of the drug (Williams, Millburn and Smith 1965; Smith 1966; Wagner 1971; Riegelman 1971). In 10 experiments with 4 different subjects the apparent biological half life ($t_{1/2}$) of conjugated 2-naphthol varied between 8 hr and 33 hr.

The present absorption studies of topically applied 2-naphthol in a so-called peeling paste, again confirm the finding of the accumulation of 2-naphthol in man reported by Lesnik and Nencki as early as 1886. Our experiments revealed a considerable percutaneous absorption of 2-naphthol, even through undamaged stratum corneum which is usually considered to be the rate limiting barrier. Treatment of the skin with a peeling paste, however, often changes the conditions by impairment of the skin barrier in such a way, that percutaneous absorption occurs even more readily (Montagna and Lobitz, 1964; Blank and Scheuplein, 1969).

A potential hazard may be created particularly in patients with widespread acne, because they, usually and preferably, are treated during a prolonged period over a relatively large fraction of the body surface. Therefore we propose that it should be made a rule that the ointment is only applied to limited areas – 300 cm^2 –, and only to patients with a normally active glucuronidating system. The patient should be followed closely during therapy with careful clinical and laboratory observations. The ointment should preferably not be left on the skin for more than one hour. A repeated treatment carried out twice a day for such a short time is preferable to one long exposure. A high urine production – preferably alkaline – must be ensured. It is obvious that pregnancy is a containdication for such a therapy.

REFERENCES

Albignente, E. (1968)
Aspetti recenti della toxicologia chimica dei fenoli
Boll Chim Farmaceutico, **107**, 269

Anonymous, (1922)
Fatal poisoning with naphthol salve
J Am Med Ass, **79**, 51.

Bakke, O. M. and Scheline, R. R. (1969)
Analysis of Simple Phenols of Interest in Metabolism
Analytical Biochem, **27**, 439

Bakke, O. M. and Scheline, R. R. (1969)
Analysis of Simple Phenols of Interest in Metabolism
Analytical Biochem , **27**, 451.

Berenbon, M. and Young, L. (1951)
Biochemical studies of Toxic Agents. 3 The isolation of 1- and 2-naphthylsulphuric Acid and 1- and 2-naphthylglucuronide from the urine of rats dosed with 1- and 2-naphthol.
Biochem. J , **49**, 165

Blank, I. H. and Scheuplein, R. J. (1969)
Transport into and within the Skin.
Br J Derm , **81**, supplement 4, 4

Boyland, E. and Wiltshire, G. H. (1953)
Metabolism of Polycyclic Compounds 7 The metabolism of naphthalene, 1-naphthol and 1 2-dihydroxy-1.2-dihydronaphthalene by Animals
Biochem J , **53**, 636.

Clarke, E. C. G. (1969)
Isolation and Identification of Drugs, 1st edition
London; The Pharmaceutical Press

Corner, E. D. S. and Young, L. (1954)
Biochemical Studies of Toxic Agents 7 The metabolism of naphthalene in Animals of different species.
Biochem J , **58**, 647.

Corner, E. D. S. and Young, L. (1955)
Biochemical Studies of Toxic Agents 8 1.2-dihydronaphthalene-1 2-diol and its role in the metabolism of naphthalene
Biochem. J., **61**, 132.

Gleason, M. N., Gosseling, R. E., Hodge, H. C. and Smith, R. P. (1969)
Clinical Toxicology of Commercial Products
Baltimore, Williams and Wilkins

Harkness, R. A. and Beveridge, G. W. (1966)
Isolation of beta naphthol from Urine after its application to Skin
Nature, Lond., **211**, 413.

Lesnik, M. and Nencki, M. (1886)
Ueber das Verhalten des alpha- und des beta-Naphthols im Organismus
Ber d D Chem Gesellsch., **19**, 1534.

Montagna, W. and Lobitz, W. C. (1964)
The Epidermis.
New York, Academic Press

Osol, A. and Farrar, G. E. (1947)
The dispensatory of the United States of America, 24th edition
Philadelphia: Lippencott.

Percival, G. H. (1967)
An introduction to Dermatology 13th edition.
Philadelphia Lippencott

Powell, E. W. (1970)
The effects of a 2-naphthol peeling paste on sebaceous glands remote from its site of application.
Br. J. Derm. **82**, 371.

Riegelman, S. (1971)
Personal communication.

Smillie, W. G. (1920)
Betanaphthol Poisoning in the Treatment of Hookworm Disease.
J. Am. Med. Ass., **74**, 1503.

Smith, R. L. (1966)
The biliary excretion and enterohepatic circulation of drugs and other organic compounds.
Progress Drug Res., **9**, 229

Van Rossum, J. M. (1971)
Significance of Pharmacokinetics for Drug Design and the planning of Dosage Regimens.
Drug Design, Vol. 1. New York-London, Academic Press

Volk, R. and Winter, F. (1936)
Lexikon der Kosmetischen Praxis.
Springer, Vienna.

Wagner, J. C. (1971)
Biopharmaceutics and relevant pharmacokinetics.
Hamilton, Illinois; Drug Intelligence Publication.

Williams, R. T., Millburn, P. and Smith, R. L. (1965)
The influence of enterohepatic circulation on toxicity of drugs
Ann. N Y Ac Scie., **123**, 110

CHAPTER III

PHARMACOKINETICS AND DISTRIBUTION OF 2-NAPHTHOL IN DOGS

SUMMARY

Plasma levels and urinary excretion data of 2-naphthol and its conjugates were measured in dogs, following intravenous administration of the drug. One dog received 480 mg 2-naphthol in 60 minutes in each of two experiments. The other dog received 200 mg 2-naphthol in 107 and 286 minutes in each of two experiments.
The concentration of 2-naphthol in the plasma in the first mentioned experiments reached a level of approximately 30 $\mu g.ml^{-1}$, and the concentration decreased rapidly after the infusion was stopped. The plasma level of the drug has been described, using a two-compartment open model. In the experiments with a lower dose of the drug, the plasma 2-naphthol level remained low, even at peak level. The plasma conjugated 2-naphthol concentration rapidly increased during the infusion. It took a long time before the conjugated 2-naphthol disappeared from the plasma. The urinary excretion rate of both 2-naphthol and conjugated 2-naphthol was non-linearly with their level in the plasma.

INTRODUCTION

Earlier studies of the percutaneous absorption of 2-naphthol in man indicate that this drug rapidly penetrates during therapeutic application to the skin (Harkness and Beveridge, 1966; This dissertation, chapter 2). An adequate study of the absorption should therefore be carried out, and, consequently, the pharmacokinetics following an intravenous dose should be defined. The toxicity of 2-naphthol has always hampered such studies. This difficulty has been overcome by administering the drug to dogs, which have as strong a glucuronidating system as man

Discussions and comments with Prof. Dr. S. Riegelman - School of Pharmacy, University of California, San Francisco - concerning this chapter have been very valuable to me.

has The course of the plasma 2-naphthol concentration following application of the drug in a peeling paste to the skin is, to some extent, mimicked by a constant-rate infusion

This chapter deals with the measurement of plasma levels and urinary excretion of the drug following such intravenous infusion in dogs Using these data, classical pharmacokinetic techniques allow an appraissal of the distribution and elimination of the drug With the aid of this knowledge it is possible to evaluate the plasma concentrations of the drug after application of the drug to the skin in different peeling pastes

MATERIALS AND METHODS

The dogs, a beagle (body weight 10 kg) and a mongrel dog (body weight 17 kg) were maintained in a sedated condition throughout the entire experiment After induction with 20 mg pentobarbital per kg body weight, they inhaled a mixture of oxygen, nitrous oxide and halothane (2 1 0 05) A parenteral solution of 2-naphthol was prepared as follows One g 2-naphthol was dissolved in 100 ml ethanol and filtered through a sterilizing filter An aliquot of this solution was added, under aseptic conditions, to a sterilized mixture of ethanol, glycerol and water (26 33 42) to a final volume of 50 0 ml Fifty ml 2-naphthol solution was delivered via a canulla to the leg vein by means of an infusion pump at a constant rate

In experiments I and II 480 mg 2-naphthol was given in 60 minutes to the beagle Two hundred mg 2-naphthol was given to the mongrel dog in 107 minutes (Experiment III) and in 286 minutes (Experiment IV)

Blood samples were taken from the jugular vein at various intervals after the start of the 2-naphthol infusion Approaching the end-point of the infusion some additional samples were taken In one experiment blood samples were additionally taken up to 200 hours after the start of the infusion The sample was collected in a heparinized test tube, centrifuged, and the plasma stored at 4° C until required for analyses

In two experiments all urine was collected from a catheter The extraction methods and the determination of free 2-naphthol and total 2-naphthol are described in chapter II

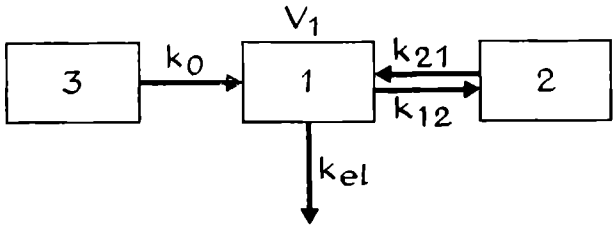
The drugs used were Dutch Pharmacopoeia Ed VI grade

PHARMACOKINETIC ANALYSIS

Individual and mean plasma 2-naphthol levels during and after the intravenous infusion of 480 mg 2-naphthol in 60 minutes were subjected

to kinetic analysis Pharmacokinetic analyses of the plasma level data was accomplished by fitting theoretical curves to the experimental data

The calculations are based on the two compartment open model, shown in the diagram,



in which 3 represents the infusion pump, 1, represents a central rapidly equilibrating body compartment which includes the plasma, 2, a more slowly equilibrating body compartment, V_1, the apparent volume of distribution of the central compartment, k_o, a fixed zero order constant (constant rate infusion) k_{12} and k_{21} are the clearance constants governing the equilibration of 2-naphthol between compartments 1 and 2, k_{el} is the overall elimination clearance constant

When the body is considered as a central compartment with one additional compartment, the semilogarithmic post infusion plasma concentration time curve is not a straight line, but is biphasic In this case (Van Rossum, 1971), the post infusion distribution and elimination processes could be described by two linear differential equations leading to the following solution

$$C_{post}(t^*) = A_1^* \; e\, t^*/\tau_1 + A_2^* \; e^{-t^*/\tau_2} \qquad \text{(Equation 1)}$$

where $C_{post}(t^*)$ equals the concentration in the first or central compartment, A_1^* and A_2^* are the coefficients - the intercepts of the exponential terms - τ_1 and τ_2 are the time constants (reciprocal rate constants) and t^* is the time after the end of the infusion i e $t^* = t - T$, where T is the infusion time

From the post infusion plasma concentration time curve A_1^* and A_2^* are obtained from the intercepts of the lines representing the slopes $r_1 (= \frac{1}{\tau_1})$ and $r_2 (= \frac{1}{\tau_1})$ with the line parrallel to the ordinate at t = 60 minutes (end of the infusion) r_1 and r_2 are obtained from the slopes by "stripping" of the post infusion curves using semilogarithmic graph

paper. The time constants, τ_1 and τ_2 in equation (1) are the same whether obtained from a post infusion curve or a single intravenous bolus curve.
During the infusion the plasma concentration time curve is describable by the following equation (Loo and Riegelman, 1970; Van Rossum, 1971; Wagner, 1971):

$$C_t = A_1 \cdot \frac{\tau_1}{T} \cdot (1 - e^{-t/\tau_1}) + A_2 \frac{\tau_2}{T} (1 - e^{-t/\tau_2}) \qquad \text{(Equation 2)}$$

where A_1 and A_2 are hypothetical intercepts with the ordinate for an intravenous bolus injection of the same amount of drug (i.e. $T = 0$). A_1 and A_2 are different from A_1^* and A_2^* (Equation 1); these differences increase as the infusion time, T, lengthens. The intercepts A_1 and A_2 have been calculated from the following equation as described by Loo and Riegelman (1970):

$$A_1 = A_1^* \cdot \frac{T/\tau_1}{1 - e^{-T/\tau_1}} \text{ and } A_2 = A_2^* \cdot \frac{T/\tau_2}{1 - e^{-T/\tau_2}} \qquad \text{(Equation 3)}$$

From these hypothetical intercepts and the time constants the pharmacokinetic parameters intrinsic to a two compartment open model have been calculated from the equations summarized in table III-1, as described by Van Rossum (1971).

Table III-1. Pharmacokinetic parameters for the two compartment open model and intravenous administration according Van Rossum (1971) *)

$A_1 + A_2 = A \qquad \tau_1 A_1 + \tau_2 A_2 = AT \qquad V_1 = D/A$

$t_{1/2} = 0\,693\ \tau_2 \qquad k_{el} = D/AT$

$r_{el} = k_{el}/V_1 = A/AT \qquad r_{21} = k_{21}/V_2 = AT/\tau_1\tau_2 A$

$r_{12} = k_{12}/V_1 = (r_1 + r_2) - (r_{el} + r_{21}) = A_1A_2 (\tau_2 - \tau_1)^2 / A\,AT\tau_1\tau_2$

$V_f = V_1 (1 + r_{12}/r_{21}) = D (\tau_1^2 A_1 + \tau_2^2 A_2) / (AT)^2$

$B_2V_2 = DA_1A_2 (\tau_2 - \tau_1) / A.AT$

---

*) In this paper the notation r is used for the elimination rate constants and the notation k for the clearance constants (Van Rossum, 1971)

The eliminated quantities Q_{el} (by metabolic, renal routes, etc.) of the drug and the quantities in the central compartment Q_1 and in the peripheral compartment Q_2 have been calculated as function of time using the following equations:

During the infusion $Q_{el(T)} = k_{el} \int_0^t C_t \, dt$ (Equation 4)

and post infusion

$Q_{el} = Q_{el(T)} + k_{el} (A_1\tau_1 (1 - e^{-t^*/\tau_1}) + A_2\tau_2 (1 - e^{-t^*/\tau_2})$ (Equation 5)

The quantity in the central compartment, $Q_1 = C_t \times V_1$ (Equation 6)

and the quantity in the peripheral compartment

$Q_2 = D - (Q_{el} + Q_1)$ (Equation 7)

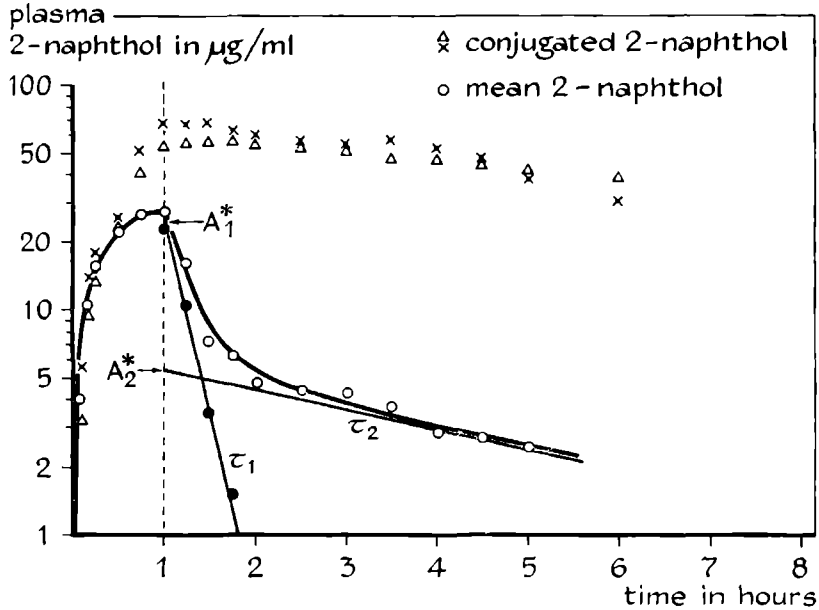


Fig. III-1 Plasma levels of 2-naphthol and conjugated 2-naphthol during and following an intravenous dose of 480 mg 2-naphthol in 60 minutes (Experiment I and II). Data points (o) represent experimentally determined mean plasma level and the continous line is the calculated level of 2-naphthol.

RESULTS

The plasma concentrations of 2-naphthol and conjugated 2-naphthol (experiment I and II) during and after constant rate infusion of 480 mg in 60 minutes are shown in figure III-1. Peak levels of 36.5 µg/ml and 22.2 µg/ml after constant rate infusion occurred at 60 minutes in both cases.
The grossly observable effect of treatment was haematuria and signs of airway irritation, with bleeding from the nose. However, soon after the end of the infusion these effects disappeared.
The kinetic constants calculated from the post-infusion plasma level are summarized in table III-2.

Table III-2. Pharmacokinetic parameters for the two-compartment open model of 2-naphthol calculated from the constant rate intravenous infusion of 480 mg 2-naphthol in 60 minutes in a beagle of 10 kg bodyweight.

Parameter		Experiment I	Experiment II	Mean value	Calculated from the mean plasma concentrations
A_1	($mg.l^{-1}$)	96	74	85	83
A_2	($mg.l^{-1}$)	7.4	7.5	7.5	6.1
A	($mg.l^{-1}$)	102.5	81.5	92.0	89.4
AT	($mg.l^{-1}.hr$)	50.4	47.6	49.0	53.2
r_1	(hr^{-1})	4.1	4.6	4.4	3.6
r_2	(hr^{-1})	0.28	0.23	0.26	0.20
τ_1	(hr)	0.25	0.22	0.24	0.28
τ_2	(hr)	3.57	4.17	3.87	4.90
V_1	(l)	4.7	5.9	5.3	5.4
k_{el}	($l.hr^{-1}$)	9.6	10.1	9.9	9.0
r_{el}	(hr^{-1})	2.0	1.7	1.9	1.7
r_{12}	(hr^{-1})	1.7	2.4	2.1	1.7
r_{21}	(hr^{-1})	0.55	0.64	0.60	0.40
k_{12}	($l.hr^{-1}$)	8.0	14.2	11.2	8.9
V_f	(l)	19.2	28.0	23.6	25.9
B_2V_2	(mg)	219	271	245	231

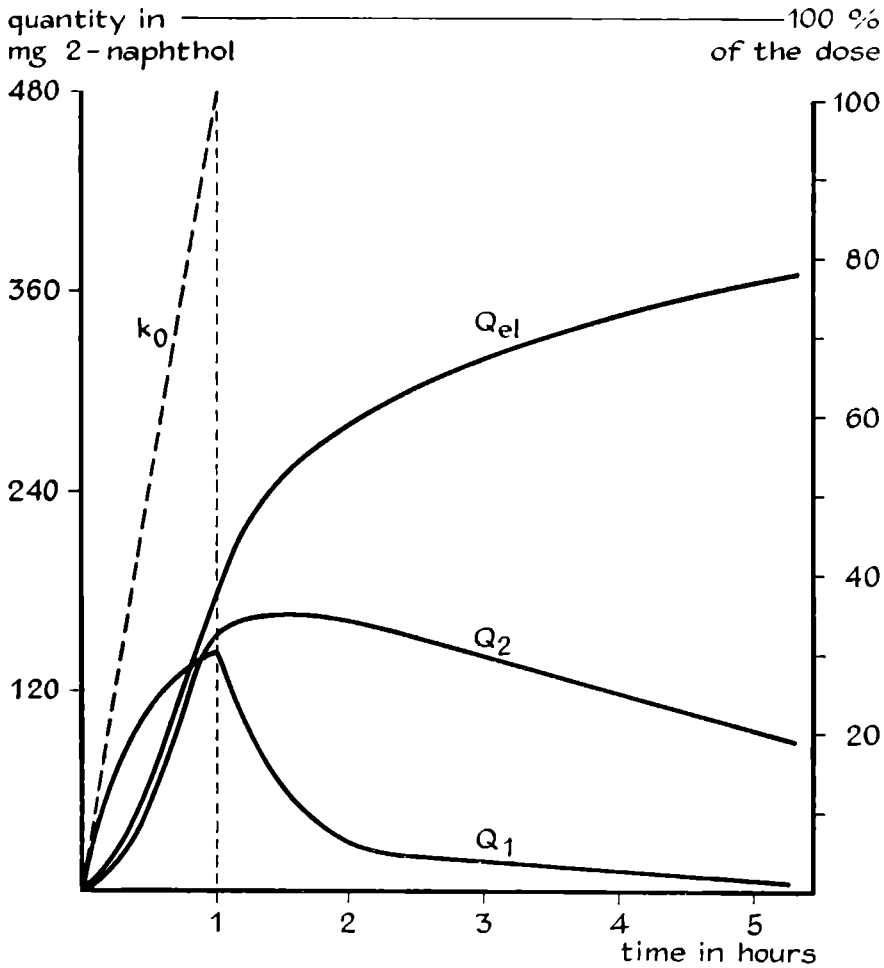


Fig III-2 Quantities of 2-naphthol in the central compartment (Q_1), the peripheral compartment (Q_2) and the quantity eliminated (Q_{el}) as function of time according to a two compartment open model and constant rate intravenous administration (k_o) of 480 mg 2-naphthol in 60 minutes to a dog of 10 kg body weight.

The continuous line in figure III-1 represents the calculated plasma level of 2-naphthol using the parameters of the mean experimental data. The quantities in the two compartments as well as the quantity eliminated calculated by using equations 4, 5, 6 and 7 from the mean experimental data are shown in figure III-2.

The results of the plasma and urine analyses during and after a constant rate intravenous infusion of 200 mg 2-naphthol in 107 minutes – Experiment III – and in 286 minutes – Experiment IV – are shown in figure III-3 and figure III-4, respectively. In comparison to the first two

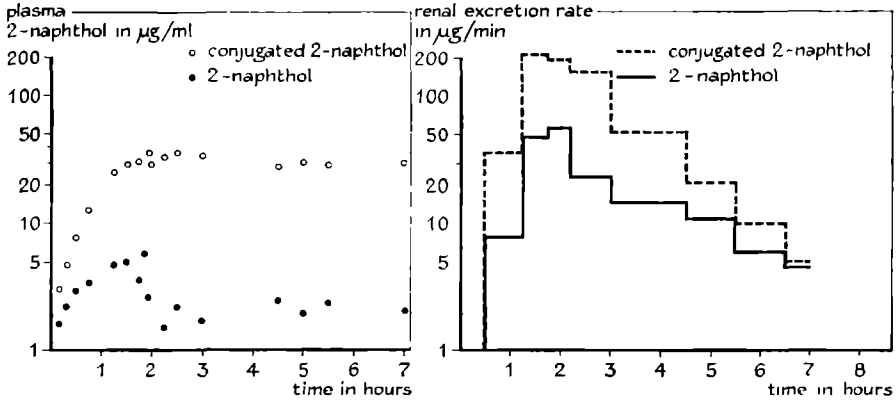


Fig. III-3. Plasma curve of 2-naphthol and conjugated 2-naphthol during and following a constant rate intravenous infusion of 200 mg 2-naphthol in 107 minutes to a dog of 17 kg (Experiment III) and the renal excretion rate of 2-naphthol and conjugated 2-naphthol during the same experiment.

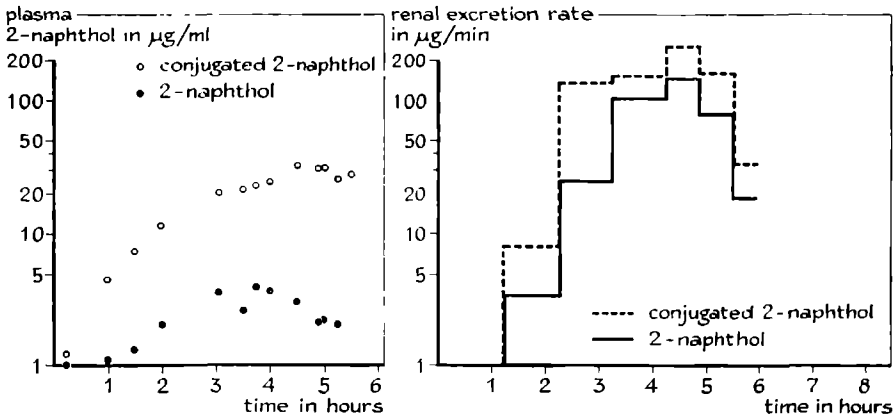


Fig. III-4. Plasma curve of 2-naphthol and conjugated 2-naphthol during and following a constant rate intravenous infusion of 200 mg 2-naphthol in 286 minutes to a dog of 17 kg (Experiment IV) and the renal excretion rate of 2-naphthol and conjugated 2-naphthol during the same experiment.

experiments the plasma 2-naphthol concentration remains low and only a brown-red urine color was observed. The plasma concentration of 2-naphthol and conjugated 2-naphthol measured during a prolonged period after the end of the infusion of 200 mg 2-naphthol in 107 minutes is shown in figure III-5 (Experiment III).

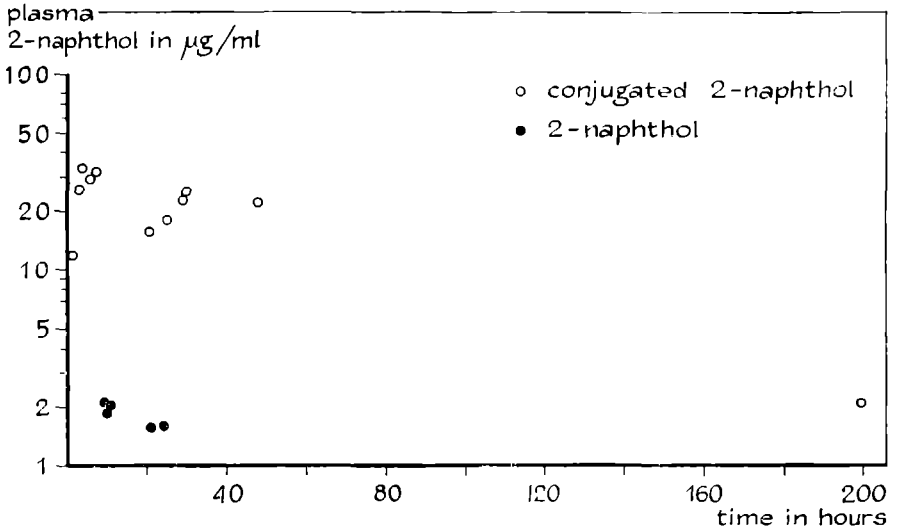


Fig. III-5 Plasma concentration of 2-naphthol and conjugated 2-naphthol measured during a prolonged period after the end of the infusion of 200 mg 2-naphthol in 107 minutes (Experiment III)

DISCUSSION

Any mathematical model used to describe a biologic system is bound to be a gross oversimplification. It is important therefore, to choose the least complicated model system which as closely as possible mimics the measurable biologic parameters With a one compartment open model, 2-naphthol plasma levels could adequately be simulated during the constant rate intravenous infusion, however, there was a difference between the calculated and observed post infusion plasma level. When the same data were analysed with the animals body described by a two compartment open model, the calculated post infusion plasma concentrations were much closer to the experimental data. Although there was still a difference between the calculated and experimental data, it was felt that any further complication of the model

was not justified without accurate analytical measurement of 2-naphthol concentrations in tissues other than the blood With the two compartment open model, 2-naphthol plasma levels could be simulated during and after stopping a constant rate infusion of 480 mg 2-naphthol in 60 minutes as shown in figure III-1

The graphical techniques used may have contributed to the differences in the pharmacokinetic parameters listed in table III-2. However, these estimates may be refined by use of an iterative nonlinear estimation program, based on the method of least squares, and a high speed digital computer.

The biological significance of some of the absolute values for the various model parameters merits comment However, due to the simplicity of the mathematical model, the rate constants and clearance constants generally reflect a combination of many processes and, therefore, are difficult to interpret On the other hand, the apparent volume of distribution of the central compartment, V_1, allows some interpretation The calculated magnitude of this parameter indicates that even in the rapidly equilibrating central compartment, 2-naphthol must be extravascularly concentrated, for the mean value of this constant (5 4 liter in the dog of 10 kg) being 0 54 l kg^{-1} is approximately 6 times the normal expected volume of the blood, being 0 09 l kg^{-1} (Jones, 1957) As for this apparent anomality, Riegelman, Loo and Rowland (1968) have concluded that highly perfused tissues such as kidneys, lung and liver might be included in the central compartment of models identical to the model used.

The total fictive volume of distribution for 2-naphthol, V_f, is even larger The drug remains in the body for a long time, notwithstanding the large clearance. It is customary to calculate the biological half-life from the largest time constant or the slowest component in the plasma curve (i e. disposition rate constant) This $t_{1/2}$ has no direct relevance to the fictive volume of distribution and elimination clearance constant

In the mentioned first experiments, the rate of infusion has to some extent exceded the rate of detoxication and excretion Observation of the dog during the experiments does suggest that the toxic level of the drug has been passed As may be seen from figure III-2,20% of the drug remains in the body 4 hours after the end of the infusion The eliminated*) 2-naphthol has not been total excreted, but most of it remains in the body as conjugated 2-naphthol

*) The term "eliminated' is used here to include that portion eliminated in the excreta as well as that part metabolized in the body

In experiments III and IV the dose was lowered in order to avoid damage to the detoxication and excretion mechanisms In both experiments the plasma 2-naphthol concentrations were markedly lower than the levels observed in the first two experiments Even at its maximum, the level is too low to allow calculations of the kinetics in the same way as in the former experiments

It is usually presumed that the excretion rate of compounds is proportional to the blood level of the compound, i e $dQ_r/dt = k_r C_t$ where dQ_r/dt is the renal excretion rate (figure III-3 and III-4), k_r the renal clearance constant and C_t, the concentration in the plasma The calculated renal clearance from the plasma levels and urinary excretion rate of experiment III (figure III-3) are summarized in table III-3

Table III-3 Relation between the plasma concentration and urinary excretion of 2-naphthol and conjugated 2-naphthol after an intravenous infusion of 200 mg 2-naphthol in 107 minutes in a mongrel dog of 17 kg - Experiment III –, see figure III-3

Time in minutes	dQr/dt in µg min^{-1}	C_t in µg ml^{-1}	k_r in ml min^{-1}
2-naphthol*)			
150	25 0	2 2	11 4
330	8 3	2 3	3 6
Conjugated 2-naphthol*)			
150	156	35 4	4 4
330	15	28 0	0 5

*) Interpolated from graphs in figure III 3

From this table, it clearly appears that both the free 2-naphthol and the conjugated 2-naphthol are excreted non-linearly with their level in the plasma The assumption of the proportionality of plasma concentration and urine excretion rate holds if there is no protein binding and provided that the urine pH is constant and that tubular secretion is concentration independent Its known from phenols, that they are highly protein bound (Deichmann, Witherop and Dierker, 1952) and most probably naphthol will behave similarly Besides, preliminary experiments showed that there was an excretion of conjugated 2-naphthol

into the gallbladder A proposed enterohepatic cycling, together with the protein binding of 2-naphthol, could be the reason why no linear relationship between the plasma level and the urinary excretion rate was demonstrable

It is generally accepted that naphthol is metabolized into its conjugates with sulphuric and glucuronic acids (Lesnik and Nencki, 1886, Berenbom and Young, 1951, Boyland and Wiltshire, 1953, Harkness, Beveridge and Davidson, 1971) The results of these studies show that the excreted conjugated 2-naphthol during the first day after the dose was mainly present in the glucuronide fraction and after the first day, the sulfate conjugate began to predominate The observation that the metabolite remaining in the plasma for a prolonged period was hydrolysed by sulfatase and not by glucuronidase confirmed this hypothesis Data obtained from the literature by Bray, Thorpe and White (1952) show that for phenols the percentage of the dose excreted as glucuronide increases with dose In our experiments the same can be concluded 8 1 mg and 0 0 mg conjugated 2-naphthol is excreted at t = 105 minutes during infusion rates of 1 9 mg min^{-1} and 0 7 mg min^{-1} respectively Glucuronide conjugation follows first order kinetics, whereas the sulfate conjugation takes place at a uniform rate (Bray, Thorpe and White, 1952, Levy and Matsuzawa, 1967; Binklley, 1949) Probably the metabolite excreted is mainly naphtholglucuronide and therefore the relationship with the composite of both metabolites in the plasma is not linear

It can be calculated from the results of experiment III and IV, that an increase in the plasma total 2-naphthol of 1 μg ml^{-1} min^{-1} corresponds to a dose of approximately 5 7 mg min^{-1} (see table III-4) Experiments

Table III-4 The relationship between the increase in plasma total 2-naphthol concentration and the infusion rate

Experiment	Dose in mg min^{-1}	Increase in plasma total naphthol in μg ml^{-1} min^{-1}	1 μg ml^{-1} min^{-1} increase in plasma total naphthol corresponds with a dose of
III	1 87	0 34	5 5 mg min^{-1}
IV	0 70	0 12	5 8 mg min^{-1}

in the same dog show that the increase in the plasma total 2-naphthol after application of 2-naphthol to the skin was a function of the surface area to which the naphthol was applied (see chapter IV fig. IV-1). This will allow comparison of data of the constant rate infusion with the percutaneous absorption experiments.

REFERENCES

Berenbom, M. and Young, L. (1951)
Biochemical Studies of Toxic Agents.
3 The Isolation of 1- and 2-naphthylsulphuric acid and 1- and 2-naphthylglucuronide from the urine of rats dosed with 1- and 2-naphthol.
Biochem J , **49**, 165

Binkley, F. (1949)
The source of sulfate in the formation of etheral sulfates.
J Biol Chem , **178**, 821.

Boyland, E. and Wiltshire, G. H. (1953)
Metabolism of Polycyclic Compounds.
7. The Metabolism of Naphthalene, 1-Naphthol and 1 2 Dihydroxy - 1 : 2 dihydronaphthalene by animals.
Biochem. J , **53**, 636.

Bray, H. G., Thorpe, W. V. and White, K. (1952)
Kinetic studies of the metabolism of foreign organic compounds
Biochem. J , **52**, 423.

Deichmann, W. B., Witherop, S. and Dierker, M. (1952)
Phenol Studies XIII. The percutaneous and alimentary absorption of phenol by rabbits with recommandations for the removal of phenol from the alimentary tract or skin of persons suffering exposure.
J Pharmacol. Exp Ther , **105**, 265.

Harkness, R. A. and Beveridge, G. W. (1966)
Isolation of beta Naphthol from Urine after its application to Skin.
Nature, Lond , **211**, 413.

Harkness, G. W., Beveridge, G. W. and Davidson, D. W. (1971)
Percutaneous absorption of 1-Naphthol (*14*C) in Man.
Br. J. Derm , **85**, 30.

Jones, S. M.
Veterinary Pharmacology and Therapeutics.
Iowa State Press, Ames, Iowa, 1957.

Lesnik, M. and Nencki, M. (1886)
Uber das Verhalten des alfa- und des beta-Napthols im Organismus.
Ber. Deutsch Chem. Ges., **19**, 1534.

Levy, G. and Matsuzawa, T. (1967)
Pharmacokinetics of salicylamide elimination in man
J Pharmacol Exp Ther **156**, 285

Loo, J. C. K. and Riegelman, S. (1970)
Assessment of Pharmacokinetic Constants from Postinfusion Blood Curves Obtained after I V Infusion
J Pharm Sci , **59**, 53

Riegelman, S., Loo, J. C. K. and Rowland, M. (1968)
Shortcomings in pharmacokinetic analysis by conceiving the body to exhibit properties of a single compartment
J Pharm Scie , **57**, 111

Van Rossum, J. M. (1971)
Significance of Pharmacokinetics for Drug Design and the Planning of Dosage Regimens
In Drug Design I, E J Ariens editor
Academic Press, New York and London (1971)

Wagner, J. C.
Biopharmaceutics and Relevant Pharmacokinetics
Hamilton, Illinois Drug Intelligence Publication, 1971

CHAPTER IV

PERCUTANEOUS ABSORPTION AND BIOPHARMACEUTICS OF 2-NAPHTHOL

SUMMARY

2-Naphthol in different ointment compositions, was applied to the flanks of an anesthetized mongrel dog Blood and urine samples were withdrawn at intervals and analyzed for 2-naphthol and its conjugates 2-Naphthol conjugates were detectable in jugular vein blood withdrawn 5 minutes after application of the drug to the flanks After a lag-time of approximately 45 minutes the conjugated 2-naphthol concentration in the plasma rose rapidly during the first hours The concentration of free 2-naphthol in the plasma remained low, i e the rate of absorption was lower than the rate of detoxication Urine 2-naphthol and conjugated 2-naphthol content were negligible during the period investigated

The penetration rate increased with increasing concentration of 2-naphthol in the ointment up to 5%, at which concentration a maximum penetration rate was reached The permeability coefficient of 2-naphthol appeared to be dependent on the concentration of the drug in each different ointment The permeability coefficient decreased with increasing concentration

Ointments with soap added reached a maximum penetration rate at approximately twice the rate reached without soap

INTRODUCTION

In in-vivo experiments percutaneous absorption is often studied by measuring the amount of material penetrating the skin by means of analysis of the amount excreted in the urine In such studies often little is known about what happens to the substance between the time it penetrates the skin and the time that it is excreted Critical remarks about such studies have frequently been made Scheuplein and Blank

(1969) remark "It is obvious that transport in such a system is a complex phenomenon and difficult to analyse"
In a previous investigation (Chapter III) the constant rate intravenous infusion technique was, therefore, applied to study the concentration of 2-naphthol reached in the plasma under defined conditions From this a model has been presented describing the results of these experiments Some processes have been explained Yet there remains a gap between the understanding of the processes involved and the practical application of 2-naphthol in the dermatological clinic
From a toxicological point of view it is important to maintain the concentration of 2-naphthol in the body and the plasma below the level which yields toxic effects The amount of penetrated substance is dependent on the nature of the penetrating molecules, the base of the ointment, the concentration, the size of the area of application and the properties of the skin These properties, that is to say the "quality" of the skin, are especially important in the clinic since a peeling ointment is applied and the skin is consequently severely injured Following the injury the systemic absorption of the 2-naphthol may become increasingly more important if the barrier due to intracutaneous absorption is nearly lost
In this chapter we describe the results after absorption of various concentrations of 2-naphthol in a peeling ointment through the skin of a dog

MATERIALS AND METHODS

A mongrel dog (17 kg) was maintained in a sedated condition as described in Chapter III The flank of the dog was shaved with an animal clipper Thirty g 2-naphthol containing ointment was applied onto an area of 18 x 23 cm^2 with a wooden spatula and the area covered with cloth The compositions of the various ointments investigated are summarized in table IV-1 The quality grade of the ingredients has been described before (Chapter II and III)
Several experiments were carried out using the same dog at 14 day intervals on alternating flanks, so that the area treated could recover during a period of a month Blood samples were drawn from the jugular vein into heparinized tubes at various intervals from the moment of application of the ointment Urine samples were taken from a catheter
The extraction methods and the determination of free 2-naphthol and conjugated 2-naphthol are described in Chapter II

Table IV-1 The composition (in % by weight) of the various ointments tested

OINTMENT nr	Percentages of			
	2-naphthol	soft soap	sulphur	soft paraffin	Total
I	1	5	20	74	100
II	3	5	20	72	100
III	5	5	20	70	100
IV	20	20	20	40	100
V	1	—	20	79	100
VI	5	—	20	75	100
VII	20	—	20	60	100

TREATMENT OF DATA

When the amount of substance penetrating the skin is plotted against time, a straight line results as soon as a steady penetration rate (r) has been reached (Treherne, 1956, Scheuplein and Blank, 1971) The value of r is obtained from the slope of the curve It has been found that an increase in the plasma total naphthol of 1 μg ml^{-1} min^{-1} corresponds to a dose of 5 7 mg min^{-1} (Chapter III and figure IV-1), and therefore this factor has been used to calculate the rate of penetration of 2-naphthol from local applications on the skin The mathematical course is less simple during the so-called "lag-time" (L) before the steady penetration rate is reached (Caneghem, 1969) The value of L has been derived from the curve by extrapolating the straight line of the steady penetration rate to an intersection with the horizontal time axis

According to Fick's law $r = dQ/dt = K\Delta C$

In this equation $r = dQ/dt$ is the rate of penetration, K is the permeability constant and ΔC is the difference in 2-naphthol concentration between the outside and inside of the skin The ΔC in the experimental circumstances can be considered to be similar to the concentration of 2-naphthol at the surface of the skin, since the 2-naphthol concentration in the blood (inner milieu) is negligible It is held at a low level by biotransformation (Chapter III) The value of ΔC is dependent on the value of the concentration of the 2-naphthol in the ointment (C_n),

as $\Delta C = f C_n$ In this equation f is the partition coefficient, representing the distribution of the 2-naphthol between the ointment and the surface of the skin Accordingly $r = K f C_n$ The product K f has been called the ' apparent permeability constant' and has been calculated from $K f = r/C_n$ The dimension is similar to the dimension of the permeability constant being calculated by dividing r in $\mu g\ cm^2\ min$ by C_n in $\mu g\ cm^3$ (derived from the percentage by weight by considering the specific gravity of the ointments, being about 0 9 g cm^3) and resulting in units of cm min^{-1}

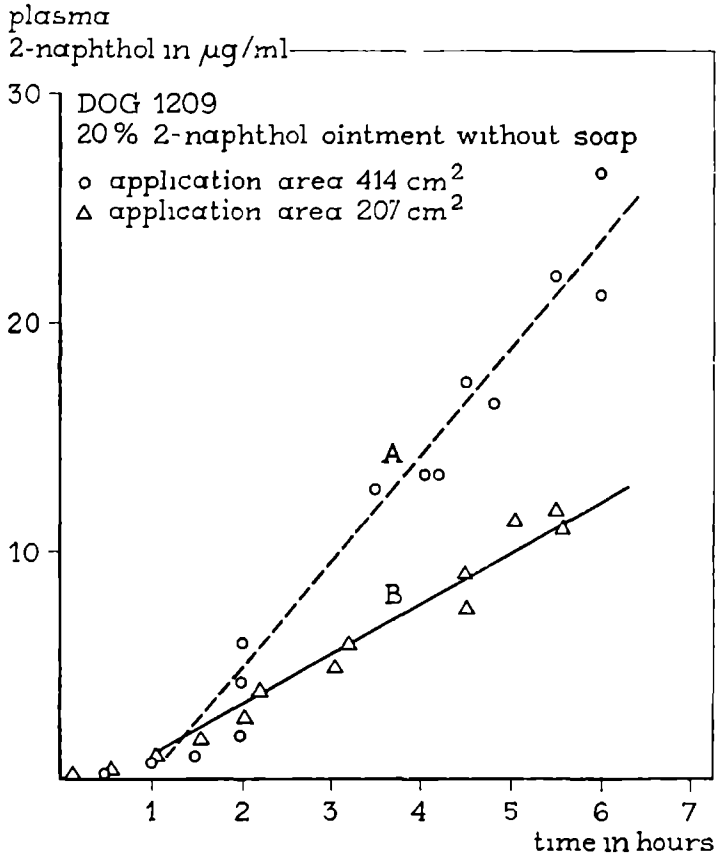


Fig IV-1 Increase in the concentration of 2 naphthol (free + conjugated)in the plasma of a mongrel dog following application of 20% 2 naphthol ointment without soap (Table IV-1 no VII) to respectively 414 cm^2 (A) and 207 cm^2 (B) skin of the flank The slope of these curves, dC_p/dt are respectively 0 08 $\mu g\ ml^{-1}\ min^{-1}$ (A) and 0 04 $\mu g\ ml^{-1}\ min^{-1}$ (B)

2-Naphthol, when applied to the shaved skin of the dog in the different ointments, was rapidly absorbed and was detectable within 5 minutes in the plasma of the blood samples obtained from the jugular vein.
The lag-time varied approximately between 30 and 60 minutes. The blood concentration of free 2-naphthol remained low throughout the experiments. The rate of absorption was below the rate of detoxication. During the investigated application times, 2-naphthol and 2-naphthol conjugates in the urine were negligible.
Typical curves for the appearance of 2-naphthol in the plasma of the dog after application of a 2-naphthol containing ointment to the flank of the dog are shown in fig. IV-1.
The mean values of the slopes of the curves (dC_p/dt) of some similar experiments are presented in table IV-2. The table also summarizes the penetration rate (r) and the apparent permeability constant (p_k) found from the results of tests with various ointment compositions.
The penetration rate of 2-naphthol in relation to the percentage of 2-naphthol in the ointment with soap as applied to the flanks of a dog is shown in fig. IV-2.

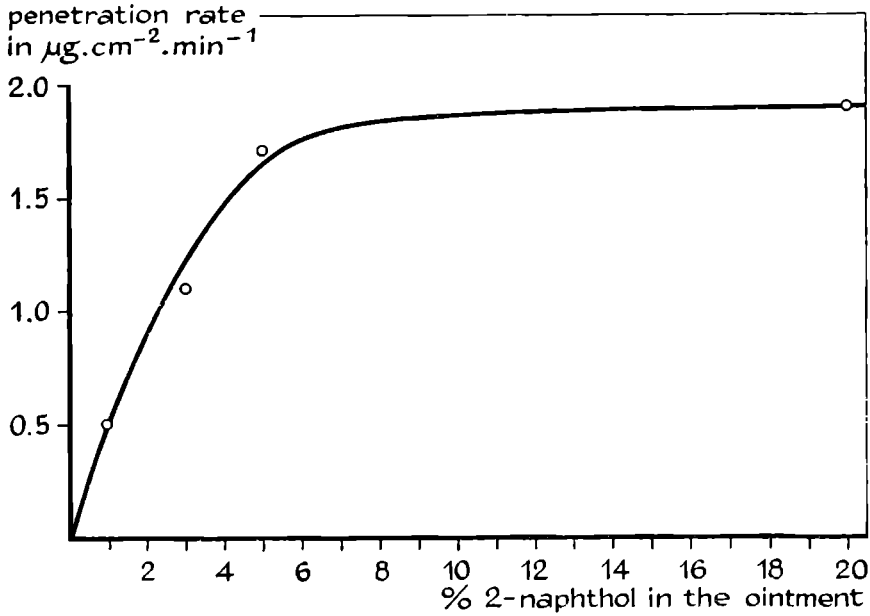


Fig. IV-2 The penetration rate of 2-naphthol in relation to the percentage of 2-naphthol in the ointment as applied to the flanks of a dog.

Table IV-2

The steady appearance rate of 2-naphthol (dC_p/dt) in the plasma of a mongrel dog (mean values ± standard deviation); the penetration rate (r); and the apparent permeability constant (p_k) following application of various ointments, mentionned in table IV-1.

OINTMENT nr	composition	dC_p/dt in $\mu g\ ml^{-1}\ min^{-1}$	r in $\mu g\ cm^{-2}\ min^{-1}$	p_k in $10^{-6}\ cm\ min^{-1}$
I	1% 2-naphthol with soap	0.04 ± 0.008	0.5	55
II	3% 2-naphthol with soap	0.08 ± 0.010	1.1	41
III	5% 2-naphthol with soap	0.12 ± 0.006	1.7	37
IV	20% 2-naphthol with soap	0.13 ± 0.009	1.9	11
V	1% 2-naphthol without soap	0.05 ± 0.007	0.7	77
VI	5% 2-naphthol without soap	0.08 ± 0.013	1.2	26
VII	20% 2-naphthol without soap	0.08 ± 0.012	1.1	6

DISCUSSION

The ability of the drug to be transported to the site of absorption, the skin, the ability of the drug to penetrate through the skin membrane (intracutaneous absorption), and the ability to penetrate from the dermal tissue into the circulatory system of the body (systemic absorption), are all important considerations (Scheuplein and Blank, 1971, Blank and Scheuplein, 1969, Hlynka, Anderson and Riedel, 1969[1], 1969[11] Idson, 1971)

In our in-vivo investigations measuring the percutaneous absorption of 2-naphthol, this absorption is effected through perhaps a damaged skin The usual barrier of the skin, the stratum corneum, can be supposed to be damaged and the systemic absorption may be of some account in these circumstances As Scheuplein and Blank (1971) have shown, it should be possible that the transport into the blood capillaries and the blood flow may not be fast enough to assure that the rate at which penetrating molecules accumulate by diffussion near to the capillary system is less than the blood perfusion rate Under such conditions the rate of systemic absorption is controlled by the transfer of molecules into the capillaries and not by diffusion through the stratum corneum of the skin The transfer resistance offered by perfusion is approximately 10^2 cm^{-1} min according to Scheuplein and Blank (1971) The diffusional resistance ($R_{diff} = 1/p_k$) calculated from the apparent permeability constants summarized in table IV-2 varied between 130 x 10^2 and 1700 x 10^2 cm^{-1} min Thus the diffusional resistance is at least about 100 times the transfer resistance offered by perfusion Evidently the percutaneous absorption of 2-naphthol is not limited by blood perfusion

For most substances the rate of penetration is limited by the impermeability of the skin Therefore at least in first instance, Fick's law is most appropriate to describe the phenomena

The amount of 2-naphthol absorbed per unit time and per unit area (the penetration rate) increased approximately linearly with increasing concentration up to about 5% 2-naphthol as might be expected from Fick's law, see figure IV-2 At a concentration of 20% 2-naphthol the absorption occurs at nearly the same rate as at a concentration of 5% 2-naphthol in the ointment however Similar apparently anomalous deviations from Fick's law have been described before in the literature in relation to the absorption of other substances (e g 1-pentanol in olive oil, mercuric chloride) through the skin (Skog and Wahlberg, 1964, Blank and Scheuplein, 1964, Wahlberg, 1968) As is evident from

table IV-2 this phenomenon finds expression in the apparent permeability coefficient, p_k, decreasing with increasing 2-naphthol concentration. The theoretical permeability coefficient, K, should be independent of the concentration. It is reasonable to suppose that the partition coefficient, f, is much influenced by the ointment composition ($p_k = K.f$). A good estimation of the partition coefficient for skin applications is not yet available.

From experiments with phenol the apparently anomalous phenomenon that some lower concentrations of phenol applied to the skin may result into higher concentrations of phenol in the blood has been described (Deichmann, Witherup and Dierker, 1952). Possibly 2-naphthol behaves similarly to some extent. A possible contribution to the understanding of this anomalous behaviour has been given by other investigators (Malten, Spruit, Boemaars and de Keizer, 1968), who demonstrated that the skin's water barrier is denaturated by phenol into a more impermeable one. It is, however, difficult to understand how such an effect should interfere in our experiments in the case of the application of the ointment composition containing 20% 2-naphthol since the skin barrier is probably thoroughly damaged by this ointment composition. The influence of soap, obvious from the different heights of the penetration level reached compared with the experiments without soap, is of the same order (2 x) as in other experiments (Bettley, 1961, 1963, 1965; Polano, 1968).

REFERENCES

Bettley, F. R. (1961)
The Influence of soap on the permeability of the epidermis
Brit. J Derm., **73**, 448

Bettley, F. R. (1963)
The irritant effect of soap in relation to epidermal permeability
Brit J Derm., **75**, 113

Bettley, F. R. (1965)
The influence of detergents and surfactants on epidermal permeability
Brit J. Derm., **77**, 98

Blank, I. H. and Scheuplein, R. J. (1964)
The epidermal Barrier.
Progress in the biological sciences in relation to Dermatology - 2 -
Editor: A Rook and R H Champion. Cambridge University Press

Blank, I. H. and Scheuplein, R. J. (1969)
Transport into and within the skin
Brit J Derm, **81**, Supp 44

Caneghem, P. van (1969)
Penetration des substances Medicamenteuses dans la peau
Louvain Med, **88**, 11

Deichmann, W. B., Witherup, S and Dierker, M. (1952)
Phenol Studies XII
The percutaneous and alimentary absorption of phenol by rabbits with recommandations for the removal of phenol from the alimentary tract or skin of persons suffering exposure
J Pharmacol Exp Ther **105** 265

Hlynka, J. N., Anderson, A. J., and Riedel, B. E. (1969)[I]
Investigations of Intracutaneous drug absorption I
Development of a quantitative Method of Measurement
Can J Pharm Scie, **4**, 84

Hlynka, J. N., Anderson, A. J., and Riedel, B. E. (1969)[II]
Investigations of Intracutaneous drug absorption II
A comparison of intracutaneous and systemic absorption as function of rest time and concentration
Can J Pharm Scie, **4**, 92

Idson, B. (1971)
Biophysical Factors in Skin Penetration
J Soc Cosm Chem, **22**, 615

Malten, K. E., Spruit, D., Boemaars, H. G. M., and Keizer, M. J. M. de (1968)
Horny layer injury by solvents
Berufsdermatosen, **16**, 135

Polano, M. K. (1968)
The interaction of detergents and human skin
J Soc Cosm Chem, **19**, 3

Scheuplein, R. J., and Blank, I. H. (1971)
Permeability of the skin
Physiol Review, **51**, 702

Skog. E., and Wahlberg, J. E. (1964)
A comparative investigation of the percutaneous absorption of metal compounds in the guinea pig by means of the radioactive isotopes
*51*Cr, *58*Co, *65*Zn *110m*Ag, *115m*Cd, *203*Hg
J Invest Derm, **43**, 187

Treherne, J. (1956)
The permeability of skin to some non-electrolytes
J Physiol, **133**, 171

Wahlberg, J. E. (1968)
Transepidermal or transfollicular absorption?
In vivo and in vitro studies in hairy and non hairy guinea pig skin with sodium (22Na) and mercuric (*203*Hg) chlorides
Acta Derm Venereol, **48**, 336

SAMENVATTING

In dit proefschrift worden de resultaten weergegeven van een onderzoek dat als voornaamste doel had informatie te verschaffen omtrent de resorptiesnelheid van naftol door de huid. Tevens had het onderzoek tot doel gegevens te verzamelen over de distributie, biotransformatie en excretie van dit geneesmiddel.
Er wordt een gas-chromatografische methode beschreven om naftol en zijn metabolieten te bepalen in plasma en urine.
Plasmaspiegels van naftol en geconjugeerd naftol zijn gemeten tijdens en na de applicatie van een 20% naftol bevattende zalf - schilpasta - gedurende de behandeling van patiënten met therapie resistente acne. Direct na de applicatie van de schilpasta is er een sterke stijging in de plasma geconjugeerd naftol concentratie. De niet geconjugeerde naftolconcentratie blijft echter laag. De uitscheiding van vrij en geconjugeerd naftol met de urine wisselt sterk, zowel bij de patiënten onderling als bij één patiënt. De verkregen gegevens tonen aan dat de stof zeer snel via de huid geresorbeerd wordt. Naast conjugatie blijkt excretie via de nier de belangrijkste wijze van eliminatie te zijn. Bij de behandeling van relatief grote oppervlakken zal de concentratie van ongeconjugeerd naftol in de tubuli van de nier zodanig stijgen dat een nierbeschadiging mogelijk is.
Om een inzicht te krijgen in de pharmacokinetiek van naftol zijn de plasmaspiegels en urinegehaltes bepaald gedurende en na intraveneuse infusies bij honden. De plasmaspiegel wordt beschreven met behulp van een twee compartimenten open model. In dit model is het plasma een onderdeel van het centrale compartiment, dat in evenwicht is met een perifeer compartiment. Evenals bij de mens wordt ook bij de hond gedurende een lange tijd geconjugeerd naftol in het plasma gevonden.
De resorptiesnelheid van naftol door de huid is na applicatie op de flanken van de hond gemeten als functie van de tijd, om de invloed van de naftolconcentratie in de zalf en de samenstelling van de zalf te meten.
Een toenemende concentratie van de naftol in de zalf geeft een stijging in de resorptiesnelheid te zien. Deze stijging bereikt bij ongeveer 5%

naftol een maximum Toevoeging van zeep aan de zalfbasis geeft een plateau dat ongeveer twee maal zo hoog ligt Voor de huid is de permeabiliteits-"constante" van de naftol in de verschillende zalven afhankelijk van de concentratie van de naftol. Bij hogere naftolconcentratie is de permeabiliteits-"constante" kleiner

STELLINGEN

I

Gelet op de verschillen die gevonden worden in de creatinine en digoxine klaring dient de dosering van digoxine gebaseerd te worden op de digoxine concentratie in het bloed en niet alleen op de creatinine klaring

G Brooker en R W Jelliffe, Circulation, 45 (1972) 20
E M Baylis, M S Hall, G Lewis en V Marks, Br Med J, 1 (1972) 338

II

De bruikbaarheid van de bepaling van de lichtverstrooiings-index van serum voor de typering van hyperlypoproteinemieen is geen argument voor de geschiktheid van deze methode voor de bepaling van serum triglyceriden

E Z Helman, E J Blevins en I O Gleason, Clin Chem, 17 (1971) 988
D A Baty en J G Batsakis, Lab Med, 2 (1971) 39

III

De conclusie van Foster et al dat propranolol geen invloed heeft op de door acetylcholine geinduceerde zweetklier response, is niet in overeenstemming met hun waarnemingen

H G W M Hemels, Br J Derm, 83 (1970) 312
K G Foster, J R Haspineall en C L Mollel, Br J Derm, 85 (1971) 363
H G W M Hemels, F A J Thiele en F W Bauer, Br J Derm, 86 (1972) 206

IV

Het is onwaarschijnlijk dat het waterdamptransport via de zweetklier in rust door middel van een uitwendige applicatie van poldine effectief kan worden geblokkeerd.

K Grice, H Sattar, M Sharratt en H Baker, J Invest Derm, 57 (1971) 108
F A J Thiele, H G W M Hemels en K E Malten, Transactions St Johns Hosp Derm Soc in press

V

Het verdient aanbeveling gedurende de therapie met naftol voor een alkalische diurese te zorgen

VI

Bij de behandeling van een groot huidoppervlak met een naftol bevattende schilpasta moet men zowel op een intoxicatie als op uitdrogingsverschijnselen bedacht blijven

VII

Het verdient aanbeveling een onderzoek in te stellen naar de sociale processen die een rol spelen bij de introduktie van geneesmiddelen en de adoptie ervan door de arts

J S Coleman, E Katz en H Menzel, Medical Innovation
New York, Bobbs-Merrill Company Inc , 1966

VIII

Het toezicht op de publieksreclame voor geneesmiddelen in Nederland dient, ook in geval van voortgaande harmonisatie van de wetgeving in E E.G -verband, opgedragen te blijven aan de K O A G G als een van de overheid onafhankelijk orgaan van *freiwillige Selbstkontrolle*

J M H J Hemels, De Nederlandse pers voor en na de afschaffing van het dagbladzegel in 1869 Diss Nijmegen 1969 stelling 3

IX

Alleen al uit economische overwegingen dient de medisch-biologische research gestimuleerd te worden

H H Fudenberg, J Lab Clin Med , 79 (1972) 353
D W van Bekkum, Intermediair 8 (1972) no 34, 15